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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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Fokke Venema

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EXAMINER

SHAW, AMANDA MARIE

ART UNIT

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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

<b>Office Action Summary</b>	<b>Application No.</b> 10/537,562	<b>Applicant(s)</b> VENEMA, FOKKE	
	<b>Examiner</b> Amanda Shaw	<b>Art Unit</b> 1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

### Status

- 1) ☒ Responsive to communication(s) filed on 29 June 2009.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

### Disposition of Claims

- 4) ☒ Claim(s) 24,25,28,29,32,33,36,37,40,41,43,44,47-52,54 and 55 is/are pending in the application.
- 4a) Of the above claim(s) 24,25,28,29,32,33,36 and 37 is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 40-41, 43-44, 47-52, 54-55 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 03 June 2005 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

### Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some \* c) ☐ None of:
- ☒ Certified copies of the priority documents have been received.
  - ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

### Attachment(s)

- |  |   |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892)                       | 4) <input type="checkbox"/> Interview Summary (PTO-413)           |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)   | Paper No(s)/Mail Date. _____                                      |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date <u>6/29/2009</u> .   | 6) <input type="checkbox"/> Other: _____                          |

### **DETAILED ACTION**

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on June 29, 2009 has been entered. This action is non final.

Claims 24-25, 28-29, 32-33, 36-37, 40-41, 43-44, 47-52, are 54-55 are currently pending.

Claims 24-25 have been amended.

Claims 24-25, 28-29, 32-33, and 36-37 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected subject matter, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on March 25, 2008.

### ***Claim Rejections - 35 USC § 102***

2. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section

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351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 40, 44, 47, and 54 are rejected under 35 U.S.C. 102(a) and (e) as being anticipated by Beckman (US 2003/0134307 Pub 7/2003 and filed 10/2002).

Regarding Claim 40 Beckman teaches molecular beacon (MB) probes comprising modified nucleotides. Specifically Beckman teaches that a MB probe comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5' end) (para 0074). In the instant case since the 5' end of the MB probe would be part of the stem region, Beckman exemplifies an MB probe comprising a stem comprising one 2'-O-methyl nucleotide and one or more unmodified nucleotides. Additionally Beckman exemplifies a probe wherein each base pair of the stem comprises no more one 2'-O-methyl nucleotide (since Beckman exemplifies that the 2'-O-methyl nucleotide is only present at the 5' end of the MB probe and the base pairs of the stem region are formed via the hybridization of the 5' and 3' ends). Further it is an inherent property of this probe that it has better stability and does not open spontaneously in the presence of contaminants present in an amplification enzyme mixture comprising said molecular beacon probe compared to a molecular beacon probe without said stem.

Regarding Claim 44 Beckman teaches a MB probe wherein the 2'-O-derivatized nucleotide is a 2'-O-methyl nucleotide (para 0074).

Regarding Claim 47 Beckman teaches that a MB probe comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5' end) (para 0074). In the instant case the 5' end of the MB probe would be part of

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the stem region and Beckman teaches that the stem regions are usually 3-25 nucleotides in length (para 0097). Therefore if the 5' end of the MB probe contained a single 2'-O-methyl nucleotide than the 5' end of the MB probe would also have 2-25 standard deoxyribonucleotides that would base pair with standard deoxyribonucleotides at the 3' end of the MB probe since Beckman exemplifies a MB probe having a 2'-O-methyl nucleotide only at the 5'end. Thus Beckman exemplifies a MB probe wherein at least one base pair constituting the stem contains no nucleotide or nucleotide analogue having an affinity increasing modification.

Regarding Claims 54 Beckman teaches a kit comprising primers, polymerase, reagents for performing amplification of an analyte, and a molecular beacon (para 0086).

### ***Claim Rejections - 35 USC § 103***

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 41, 43, 48-52, and 55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Beckman (US 2003/0134307 Pub 7/2003 and filed 10/2002) in view of Majlessi (Nucleic Acids Research 1998) and Tsourkas (Nucleic Acids Research 2002).

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The teachings of Beckman are presented above. It is reiterated that Beckman teaches that a MB probe can comprise one or more 2'-O-methyl nucleotides or a MB probe can consist entirely of 2'-O-methyl nucleotides (para 0074).

Beckman does not specifically teach a MB probe wherein said loop comprises one or more 2'-O-methyl nucleotides wherein the sensitivity of said probe to polymorphisms in the target nucleic acid sequence is lowered as compared to a MB probe without said loop (clm 41). Additionally Beckman does not specifically teach a MB probe wherein one base pair constituting the stem contains no 2'-O-methyl nucleotide (clms 48-50). Beckman does not specifically teach a MB probe wherein each strand constituting the stem contains at least one 2'-O-methyl nucleotide (Clms 51 and 52). Further Beckman does not specifically teach a kit comprising a MB probe as claimed in claim 41 (clm 55).

However Majlessi teaches that 2'-O-methyl oligoribonucleotide probes afford multiple advantages over 2' deoxy oligoribonucleotide probes for detecting RNA targets, including greatly increased  $T_m$  which allows use of shorter probes, faster kinetics of hybridization, ability to bind to structured targets under conditions where 2' deoxy oligoribonucleotide probes will not and significantly improved specificity. Majlessi further states that these advantages render 2'-O-methyl oligoribonucleotide probes superior to 2' deoxy oligoribonucleotide probes for use in assays that detect RNA targets (page 2224 and 2229). Thus the benefits of using 2'-O-methyl modified probes were well known in the art at the time of the invention.

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Additionally Tsourkas teaches that 2'-O-methyl oligoribonucleotides bind RNA with higher affinity and faster kinetic hybridization rates, resist nuclease degradation, and do not form a substrate for RNase H. Tsourkas further teaches that 2'-O-methyl MB probes form a more stable stem-loop structure because of the presence of the 2'-O-methyl nucleotides. In the absence of target, the 2'-O-methyl MB exhibited a higher  $T_m$  and a lower level of background fluorescence compared with the 2' deoxy MB. The 2'-O-methyl modification of the MB backbone resulted in a higher affinity for target mRNA. The melting temperature of the 2'-O-methyl/RNA hybrid was found to be significantly higher than that of the 2'-deoxy/RNA hybrid (page 5173). Thus the benefits of using 2'-O-methyl modified probes were well known in the art at the time of the invention.

While Beckman does not exemplify each and every probe recited by the claims designing probes which are equivalents to those being claimed is considered routine experimentation especially since MB probes comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides had already been described by Beckman. Further the advantages of using probes comprising 2'-O-methyl nucleotides were already known in the prior art and are taught by Majlessi and Tsourkas. Although Majlessi and Tsourkas compared probes consisting of 2'-O-methyl oligoribonucleotides to probes consisting of 2' deoxy oligoribonucleotides one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2' deoxy oligoribonucleotides would also be useful. Thus the prior art is replete with guidance and information necessary to permit the ordinary artisan to design MB probes that have better stability and do not open spontaneously (because the stem region comprises 2'-

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O-methyl nucleotides) and probes that are more sensitive to polymorphisms (because the loop region comprises 2'-O-methyl nucleotides). Based on the computer programs available an ordinary artisan would have had more than a reasonable expectation of success of designing the probes that have better stability and do not open spontaneously and probes that are more sensitive to polymorphisms. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

### ***Response To Arguments***

4. In the response filed June 29, 2009, Applicants traversed the rejection made under 35 USC 102. The Applicants agree that Beckman teaches that the MB probes are either made entirely of 2'-O-methyl nucleotides or comprise one or more 2'-O-methyl nucleotides at the 5' end of the MB probe (para 0074). However, Applicants argue that Beckman does not clearly state which of the three 5' ends of the probe is being referred to: the first or second arms (stems) or the loop.

This argument has been fully considered but is not persuasive. In the instant case there is nothing in the Beckman reference that would lead one to think that there is more than one 5' end of a MB probe. Beckman specifically teaches "a MB comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5' end)" (para 0074). In view of the word "**end**", opposed to "**ends**", it clearly suggests that there is only one end. Further Beckman teaches that a MB probe can



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have a fluorophore on the 5' end and quencher on the 3' end (para 0004). As shown in Figure 9A the fluorophore is located on the 5' end of the MB probe as a whole and the quencher is located on the 3' end of the MB probe as a whole. Therefore the phrase "5' end" is being interpreted as the 5' end of the MB probe as a whole.

Next the Applicants state that one of ordinary skill in the art would understand, 5' nuclease domains of DNA polymerases (e.g., Taq) "specifically recognize bifurcated ends of double stranded regions and remove single stranded 5' arms by cutting the phosphodiester bond after the first base pair of the duplex, leaving a 3' hydroxyl end." (Kaiser et al. J. Biol. Chem. 274:21387-21394 (1999), page 21387, first paragraph; copy enclosed) (See also, Lyamichev et al. Science 260:778-783 (1993), page 779, first paragraph and Figure 1; and Huang et al. Mol. Cell. Probe 23:188-194 (2009), page 193, last sentence; copies enclosed). Applicants believe that in order to block nuclease activity, the MB probes must have the modified nucleotide at the 5' end of the loop region, where the hybridization of the probe with the target nucleic acid creates a bifurcated double stranded molecule that can be recognized by the nuclease region of the polymerase. Applicants argue that having a modified nucleotide at the 5' end of the stem region as suggested in the Office Action would have no blocking effect on the nuclease activity because this is not the recognition site or site of cleavage for the enzyme. Therefore, Applicants assert that Beckman does not teach one or more 2'- O-methyl nucleotides in the stem of a MB probe.

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This argument has been fully considered but is not persuasive. Beckman teaches nuclease resistant MB probes (para 0074). In one embodiment Beckman teaches a nuclease resistant MB probe that comprises standard deoxyribonucleotides and one or more 2'O-methyl nucleotides at the 5' end (para 0074). Based on the papers cited by the Applicants the examiner agrees that a MB probe with a single 2'O-methyl nucleotide at the 5' end of the MB probe as a whole would not be able to block the nuclease activity of Taq polymerase (which has 5' to 3' endonuclease activity (see para 0037)). However a MB probe with a single 2'O-methyl nucleotide at the 5' end of the MB probe as a whole would be able to block the activity of a true 5' exonuclease. Here it is important to note that the term "nuclease" encompasses both exonucleases and endonucleases. Beckman teaches that the phrase "5' to 3' nuclease activity" includes either a 5' to 3' exonuclease activity, whereby nucleotides are removed from the 5' end of a nucleic acid strand in a sequential manner; or a 5' to 3' endonuclease activity, wherein cleavage occurs more than one nucleotide from the 5 end; or both (para 0037). As such a MB probe with a single 2'O-methyl nucleotide at the 5' end of the MB probe as a whole would still be considered to be a nuclease resistant MB because it would be resistant to 5' exonucleases.

The Applicants also traversed the rejection made under 35 USC 103 over the combination of Beckman, as evidenced by Majlessi and Tsourkas. First the Applicants reiterated why they believe that Beckman does not teach a MB probe comprising a stem comprising one or more nucleotides or nucleotide analogues having an affinity increasing modification, wherein said one or more nucleotides or nucleotide analogues

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are selected from the group consisting of a 2'-O- derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2' -O- methyl nucleotide.

Applicant's arguments regarding the Beckman have been fully addressed above. The response to Applicants arguments as set forth above applied equally to the present grounds of rejection.

Next the Applicants state that the previous Office Action provides no evidence to support the allegation that one of skill in the art would have recognized that probes consisting of both 2-O-methyl nucleotides and 2'-deoxy nucleotides would also be useful or the allegation that the prior art is replete with guidance and information to permit the ordinary artisan to design MB probes that have better stability, do not open spontaneously and are more sensitive to polymorphisms.

The Applicants state that Majlessi compares linear probes of different lengths and does not teach or suggest MB probes or solutions to problems associated with the premature opening, stability or specificity of such probes. Further Applicants state that Majlessi does not teach or suggest probes having a combination of 2'-O-methyl nucleotides and 2'-deoxy nucleotides for any purpose, much less to improve sensitivity to polymorphisms in the target nucleic acid or to prevent the premature opening of a MB probe as is claimed in the present invention. Therefore Applicants argue that Majlessi fails to provide any teaching or suggestion that would motivate one of skill in the art to combine Majlessi with Beckman so as to produce a MB probe comprising a stem comprising one or more nucleotides or nucleotide analogues having an affinity

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increasing modification, wherein said one or more nucleotides or nucleotide analogues are selected from the group consisting of a 2'-O-derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2' -O-methyl nucleotide.

This argument has been fully considered but is not persuasive. In the instant case Majlessi is not being relied upon to teach a MB probe comprising a stem comprising one or more nucleotides or nucleotide analogues having an affinity increasing modification, wherein said one or more nucleotides or nucleotide analogues are selected from the group consisting of a 2'-O-derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2' -O-methyl nucleotide because Beckman already teaches this. Majlessi is being relied upon to demonstrate that it was well known in the art at the time of the invention that there were advantages to using probes comprising 2'-O-methyl oligoribonucleotides over 2' deoxy oligoribonucleotides. The Examiner acknowledges that Majlessi refers to linear probes however the reference is relevant to the instant invention. Majlessi teaches that 2'-o-methyl oligoribonucleotide probes bound to RNA targets faster and with much higher melting temperatures than corresponding 2'-deoxyribonucleotide probes (abstract). This teaching is important because it describes the base pairing of a 2'-o-methyl nucleotide and a unmodified nucleotide which is required by the present claims since each base pair in the stem comprises no more than one 2'-o-methyl nucleotides. Further the fact that Majlessi does not teach probes having a combination of 2'-O-methyl nucleotides and 2'-deoxy

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nucleotides is irrelevant because Beckman clearly teaches MP probes that have a combination of 2'-O-methyl nucleotides and 2'-deoxy nucleotides.

The Applicants state that Tsourkas describes MB probes consisting entirely of 2'-O-methyl nucleotides or consisting entirely of 2' deoxy nucleotides. The Applicants state that Tsourkas teaches that MB probes consisting of 2'- O-methyl nucleotides have a reduced ability to discriminate between the wild-type target and the mutant target nucleic acids (page 5169, first column, first full paragraph, last sentence). Applicants argue that one of ordinary skill in the art reading Beckman and Tsourkas and desiring to increase the sensitivity of a probe to detect polymorphisms would be lead away from substituting unmodified nucleotides with modified nucleotides because Tsourkas teaches that this would in fact reduce sensitivity. Further, Applicants argue that one of ordinary skill in the art reading Beckman as evidenced by Tsourkas and interested in improving a MB probe for diagnostic assays would not consider combining an unmodified nucleotide with a modified nucleotide in a base pair in the stem as claimed herein because such a skilled person would understand from the prior art that this would decrease the stability of the MB probe. Specifically Applicants state that Tsourkas teaches away from such a MB probe in the statement that "...2'-O-methyl molecular beacons form a more stable stem-loop structure because of the 2'-O-methyl/2'-O-methyl interactions " (Tsourkas et al., page 5173, first column, last sentence).

This argument has been fully considered but is not persuasive. Tsourkas is being relied upon to demonstrate that it was well known in the art at the time of the invention that there were advantages to using MB probes comprising 2'-O-methyl

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nucleotides over unmodified nucleotides. Tsourkas teaches that “We found that the 2'-o-methyl molecular beacons hybridize to RNA more quickly and with higher affinity than 2'-deoxy molecular beacons even though they exhibit a much more stable stem-loop structure. However, the enhanced affinity between 2'-o-methyl molecular beacons and RNA is accompanied by a slightly reduced ability to discriminate between wild type and mutant targets”. The argument that one desiring to increase the sensitivity of a probe to detect polymorphisms would be lead away from substituting unmodified nucleotides with modified nucleotides is misleading because Tsourkas merely states that there is a trade off. While there may be a slightly reduced ability to discriminate one would gain the added benefit of faster hybridization and a higher affinity. As such one of skill in the art would not necessarily be lead away from substituting unmodified nucleotide with modified nucleotides. Further, the argument that one interested in improving a MB probe for diagnostic assays would not consider combining an unmodified nucleotide with a modified nucleotide in a base pair in the stem because this would decrease the stability is not persuasive. While there may be a reduced stability one would gain the added benefit of faster hybridization and a higher affinity. As such one of skill in the art would not necessarily be lead away from substituting unmodified nucleotide with modified nucleotides. Additionally it is noted that the prior art of Beckman teaches combining an unmodified nucleotide with a modified nucleotide in a base pair in the stem.

The Applicants state that they have surprisingly discovered that the designing of a MB probe having better stability that does not open spontaneously depends not only

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on the presence of nucleotide analogues in the stem but also on the number of nucleotide analogues, their position in the stem or loop of the MB probe and the sequence of the stem or loop of the MB probe. The Applicants refer to, Table 6 of Example 4 which shows that the MB4 probe having all modified nucleotides has a greater percentage of spontaneous opening (IBL- Increase of Baseline) than reference MB which is comprised entirely of unmodified nucleotides. The MB4 probe having all modified nucleotides also has a greater percentage of spontaneous opening as compared to MB probes comprising a combination of unmodified and modified nucleotides. Furthermore, as shown by the other MB probes provided in Table 6, the position and number of nucleotide analogues in the probe are clearly shown to affect rates of spontaneous opening. None of the cited art teaches or suggests that the content and placement of the modified nucleotides in an MB probe would play an important role in the functional features of the MB probe.

This argument has been fully considered but is not persuasive. As discussed above Beckman teaches a MB probe wherein each base pair of the stem comprises no more one 2'-O-methyl nucleotide (since Beckman exemplifies that the 2'-O-methyl nucleotide is only present at the 5' end of the MB probe and the base pairs of the stem region are formed via the hybridization of the 5' and 3' ends). The MPEP 2112.01 states that where the claimed and prior art products are identical or substantially identical in structure the claimed properties or functions are presumed to be inherent. As such it is a property of the MB probe of Beckman that it has better stability and does not open spontaneously.

***Conclusion***

5. No Claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Amanda M. Shaw whose telephone number is (571) 272-8668. The examiner can normally be reached on Mon-Fri 7:30 TO 4:30. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Doug Schultz can be reached at 571-272-0763. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Amanda M. Shaw  
Examiner  
Art Unit 1634

/Carla Myers/  
Primary Examiner, Art Unit 1634